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# Behavioral Changes of *Heterotermes indicola (*Isoptera: Rhinotermitidae*)* Against Some Natural Products

**Ahmad-Ur-Rahman Saljoqi,[1] * Muhammad Anwar Khan,[1] Zell-e-Huma,[1] Abdus Sattar,[2] Misbah Ullah[2] and Faheem Khan[3]**

[1]*Department of Plant Protection, Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan*
[2]*Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan*
[3]*Department of Extension Education and Communication, Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan*

**Abstract.-** Study regarding the response of five different plants extracts *viz.* garlic *(Allium sativum),* turmeric *(Curcuma longa),* black tea (*Camellia sinensis*)**,** green chilies (*Capsicum annum*) and ginger *(Zingiber officinale)* on termite species *Heterotermes indicola* was conducted at Nuclear Institute for Food and Agriculture (NIFA), Peshawar. Garlic, ginger and green chilies were used with the ratio of 1:2 and tea and turmeric in 1:4 (W/V). After one day of application garlic caused significantly high mortality (100%) compared to chilies, turmeric, ginger and tea, while 100% mortality occurred in chilies, turmeric, ginger and tea after $9^{th,}$ $10^{th,}$ and $11^{th}$ day, respectively. Termiticidal effect of all treatments showed that the most toxic treatment was garlic followed by green chilies, turmeric, ginger and black tea. Behavioral response were studied using Abid's track move software with respect to control. Total distance covered in all treatments was reduced as time passed while in turmeric the total distance covered was increased. Average speed covered by termite worker was also different in all treatments. The speed of termite worker was increased in turmeric compared to that of ginger, black tea, garlic and green chilies. Pause time and non pause time effect was found different in turmeric compared to black tea, ginger, garlic and green chilies. Resting periods in all treatments were increased or their movement slowed down except in turmeric in which termite worker became excited as the time passed.

**Key Words:** Behavioral changes, plant extracts, *Heterotermes indicola*

## INTRODUCTION

Termites are widely distributed throughout Pakistan and especially in Khyber Pakhtunkhwa. They are either soil or wood inhabiting termites (Chaudhry *et al*., 1972). *Microtermes mycophagous, Microtermes obesi, Microtermes unicolor, Eremotermes paradoxalis* and *Odontotermes obesus* are the species mostly recorded from agro-ecosystems of Pakistan (Ahmed *et al*., 2004). Termites like *Odontotermes, Heterotermes* and *Coptotermes* have been observed to infest and cause damages to apricot, pear, plum, peach, orange and lemon (Salihah *et al*., 1994). The most troublesome type of termite in agriculture is the fungus-growing termites. They feed on dead organic material such as crop residues, mulches and soil organic matter (humus). However, when this type of food is not available they attack live plant material including crops such as groundnuts, millets, maize and sugarcane. At germination stage, losses up to 90-100% have been recorded in sugarcane due to termite attack (Salihah *et al*., 1988).

*Heterotermes* spp. are structure-infesting termites that account for a significant proportion of damage attributed to subterranean termites, wherever it occurs. The termite genus *Heterotermes* Froggatt has centers of abundance and diversity in the warm Neotropics, Australia, the Indian subcontinent, and the Arabian Peninsula. It is also reported that individual species of *Heterotermes* are confined to their respective climatic zones by the limits of soil moisture and temperature (Emerson, 1971). *Heterotermes* spp are widespread termites on the mainland (Constantino, 1998, 2000). Three species of Neotropical *Heterotermes* are reported from the West Indies (Snyder, 1924, 1949; Constantino, 1998).

Many control measures have been adopted to control termites, among which chemicals were dominant means of termite control for long time,

* Corresponding author drsaljoqi@yahoo.com; Phone: 091-9216554; Cell: 03339139959; Fax: 091-9216520
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however, chemicals are expensive and have many harmful effects on human health. The main focus of previous studies has been on chemical control without understanding behavior and natural history of termites. Insecticides treated soil barrier have been widely used for subterranean termite control for the past 50 years (Forschler, 1998). The objective of termiticide soil treatments is the exclusion of termites from structures in ground contact. Consequently, termiticides applied to establish soil barriers can be repellent, toxic, or both (Forschler, 1994). Numerous chemicals have been investigated, and their effects vary with environmental factors or soil conditions, which affect the residual activity and longevity of biocides (Smith and Rust, 1993; Forschler and Townsend, 1996).

The injudicious use of pesticides against termites has generated a number of biological and environmental hazards in air, water, soil and food, which have further resulted in phytotoxicity, mammalian toxicity, pesticides residues, insect resistance, insect outbreaks and increased cost of production. The direction being taken by many researchers is towards alternative non-toxic and biological methods of control. These newer methods that are being investigated include baiting, asphyxiant gases, extreme temperatures, barriers of various types, and biological control organisms (Lewis, 1997).

There has been recent interest in plant-derived compounds as alternatives to synthetic insecticides in pest control programmes (Gonzales-Coloma *et al.*, 1994; Addor, 1995). Natural resistance of different wood species to termite infestation was reported decades ago (Wolcott, 1947; Sandermann *et al.,* 1958; Hrdy, 1961). Plant parts and plant extracts can be used effectively because these are less expensive, biodegradable and hence environmentally suitable.

Many farmers in Asia and Africa had been using plant extracts such as neem, wild tobacco, dried chillies, Ak (*Calotropis procera)* and wood ash etc. for controlling and repelling termites (Anonymous, 2000). Many plants have been found to contain chemicals but their potential has not been explored for field use (Robert, 2001; Zhu *et al*., 2001). The reduction in infestation of termites in the sugarcane with neem and *calotropis* sp. extracts has been reported (Singh *et al*., 2002; Sohail *et al*., 2005). The chemical poisons of plants are mostly alkaloids, which are plant products and nitrogenous in nature, having strong effect on the nervous system of animals. The alkaloidal extracts when applied to the insects bring about disturbance in the nervous system and cause death (Okugawa *et al*., 1993). Several attempts have been made to use plant toxicants, which are eco-friendly and also play a better role in the control of these pests (Lin and Wang, 1988; Haung *et al.,* 1990; Parihar, 1994; Hutchins, 1997).

The present study aims at testing local natural products for their toxicological characteristics against *H. indicola* and to find out the behavioral changes in *H. indicola*.

## MATERIALS AND METHODS

*Termites culture*

Termites were collected from termite-infested orchards/building by using trapping technique developed by Salihah *et al.* (1994). Wooden stakes (2.5×4.0×28 cm) made of poplar wood were driven 25 cm deep into the soil in grid pattern (2.4×2.4meter) around building and in orchards and were observed fortnightly. Active foraging points were marked and infested stakes were replaced by NIFA-TERMAPS. After 15 days the infested bundles of NIFA-TERMAPS were brought to the laboratory of entomology division NIFA Peshawar to separate termites from soil and other debris. Termites were identified with the help of the taxonomic keys (Chaudhry *et al.*, 1972). The termites, *H. indicola,* were cultured in the lab on blotting paper in petri dishes and placed in desiccators at 25±2ºC and relative humidity at 68±2%, for experimental purpose.

*Plant extracts/natural plant product preparations*

Garlic *(Allium sativum)*, turmeric *(Curcuma longa)*, black tea (*Camellia sinensis*), green chilies (*Capsicum annum*) and ginger (*Zingiber officinale)* were cleaned, chopped with pestle and mortar and then soaked in distilled water in a ratio of 1:2 (W/V) while turmeric and tea were mixed in the ratio of 1:4 (W/V) separately and the mixture/solution kept for

24 h at ambient temperature. The extracts were filtered through muslin cloth and used for experimentation.

*Bioassays*

For bioassays six treatments including control were used. A completely randomized design was used wherein each treatment was replicated three times. Each experimental unit for all treatments consisted of two blotting papers, a petri dish and termites. Two blotting papers of petri dish size were dipped in a plant extract and then were placed in petri dishes separately for each treatment. Just after soaking, 25 workers of *H. indicola* were released in each petri dish. These dishes were then shifted to desiccators having water at its bottom to maintain 70% humidity and controlled room temperature of 27-30 ºC. The termites were allowed to feed and data was recorded after every 24 h till 100 % mortality of termites was achieved in all the treated dishes. Data were analyzed using Proc ANOVA in SAS (SAS Institute, 1985). Duncan Multiple Range Test (DMR) was used for means separation.

*Termite behavior or response*

Insect behavior to different plant extracts was recorded using the software Abid's Track move. The software calculates total distance, average speed, pause time and non pause time of the subject individual. (*http://www.nifa.org.pk/Trackmove.htm*).

In order to mark the experimental arena a grid printed on transparent plastic sheet (15×13.5 cm) having each square of size 1.5 × 1.5 cm was placed on the cover of each petri dish (14 cm diameter).

A circular blotting paper (to fit the bottom of petri dish size) dipped in natural plant product/ treatments (prepared a day before) was placed in petri dish. A single termite worker was released and its walking behavior was recorded soon after its release using the software and that time was considered as 0 mints. The second and third observations were then recorded after five and ten minutes. Each observation time was three minutes. The data recorded for each plant product was compared with control in which distilled water was used for soaking blotting paper. The same worker was used for control and the treatment. A total of three replicates were used each represented by a different worker. In order to make the comparisons, the data for each treatment was adjusted against the control using the following formula:

$$\text{Adjusted response} = ((\text{Treatment} - \text{control}) \div \text{control}) \times 100$$

The sign of the resulting adjustment (+ or -) indicated a percent increase or decrease in that parameter as compared with the control.

## RESULTS

*Toxic effects of natural products*

Results on the effect of different plant extracts against *H. indicola* are presented in Figure 1. On day 1, percent mortality due to application of garlic extract was significantly higher from other treatments including control. The mortality in garlic was 100% after first day, while there was no termite mortality in control as well as in black tea extract. At the same time, mortality due to ginger extract was only 2%, which was statistically not different black tea extract and control. Mortality due to green from that of chilies and turmeric extract was 3.33% which was not different from that of ginger but was significantly higher than in black tea and control.

On second day, the mortality due to green chilies and turmeric extract was 4.67% which was significantly higher than control in which no mortality occurred. Mortality in ginger and black tea extract was statistically same to green chilies, turmeric extract which was 3.33% and also to control which was1.33%.

On third day, the percent mortality due to green chilies extract was significantly higher (8.67%) from ginger and black tea extract. In ginger and black tea extract the mortality was 5.33% and 4.67%, respectively, which was statistically similar to turmeric extract having mortality of 7.33%. Mortality was significantly low in control when compared with other extracts.

On fourth day, results showed that in control there was no mortality, which was significantly different from green chilies extract with 46.67% mortality. Mortality in turmeric and ginger extract was statistically non significant to green chilies with 20% and 16.67%, respectively, and at the same time with black tea having mortality of 6.67% and

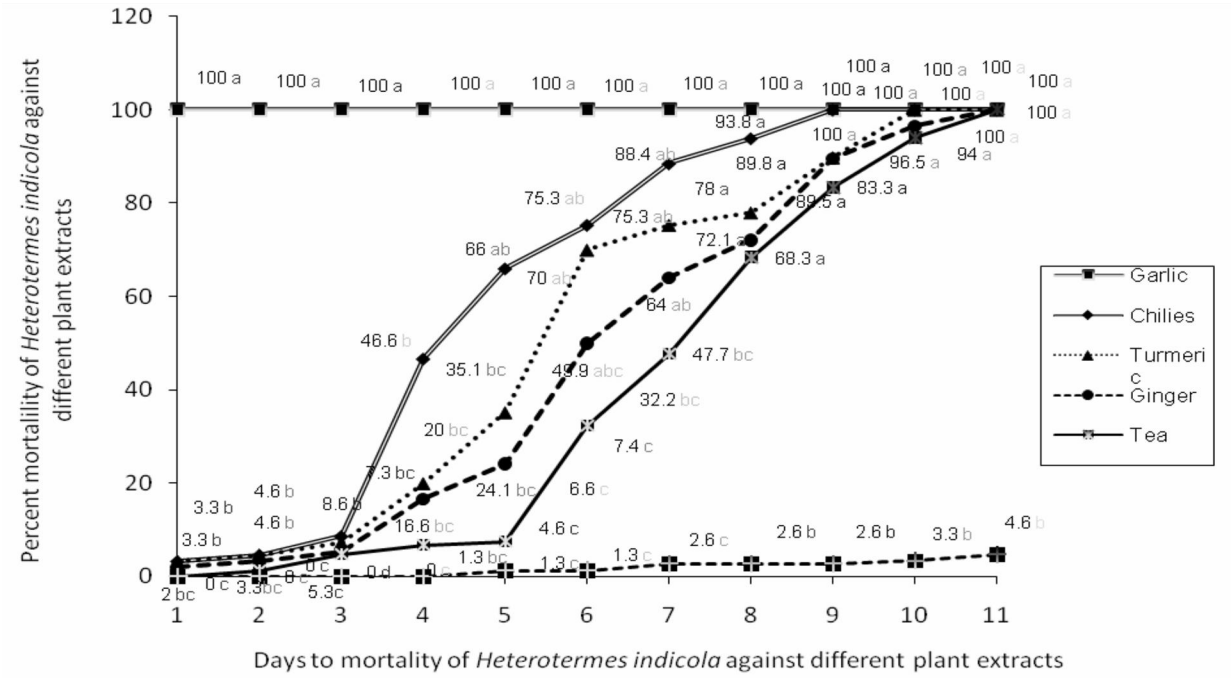


Fig. 1. Effect of different plant extracts on percent mortality of *Heterotermes indicola.* Mean followed by the same letter in same day r non significant with each other at 5% level of significant.

control with no mortality. Mortality caused by black tea and control was statistically same garlic, chilies and tea extracts caused mortality which was statistically significant with each other but statistically non significant with turmeric and ginger extracts.

On fifth day, mortality due to green chilies extract was 66%, which was statistically non significant to garlic as well as to turmeric and ginger extracts with 35.19% and 24.17% mortality respectively. The result of black tea extract and control was statistically similar with 7.42% and 1.33% mortality, respectively, and non significant to turmeric and ginger extract.

On sixth and seventh day, mortality due to garlic extract was significantly different from control but same to green chilies, turmeric, ginger and black tea extract. Mortality in garlic extract was 100% while that in green chilies, turmeric, ginger and black tea extract it was 75.33, 70, 49.97 and 32.28%, respectively on day sixth and 88.49, 75.33, 64.07 and 47.91%, respectively on seventh day. Mortality in control on day sixth was 1.33% and 2.67% on day seventh.

Day eighth results of all treatments were found statistically same as compared to control which was 100, 93.88, 78, 72.11 and 68.34% in garlic, green chilies, turmeric, ginger and black tea respectively. Mortality in control was only 2.67%.

Results of ninth day showed that 100% mortality was recorded due to green chilies extract while due to turmeric extract this mortality on next day. In control mortality was only 3.33% on tenth day, which reached to 4.67% up to eleventh day.

*Termite response*

The total distance covered by termite worker under the influence of natural products compared to control is presented in Figure 2. Results revealed that at 0 min after release, the total distance covered by termite worker was 40.14% less than that in control in case of turmeric extract, while it was 4.77, 17.20, 21.52 and 26.16% less in ginger, black tea, garlic and green chilies extract, respectively, compared to control. After 5 min the speed of termite worker became slow and ultimately total distance covered by it was reduced in all treatments except turmeric. In ginger extract it was 31.89% less

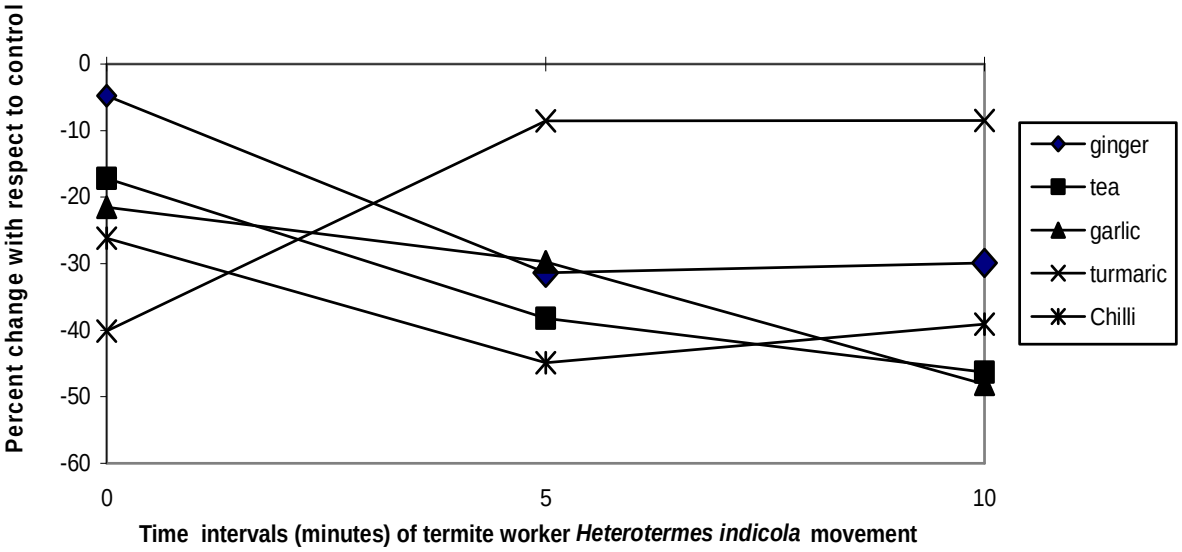


Fig. 2. The effect of different plant extracts on total distance covered by termite worker.

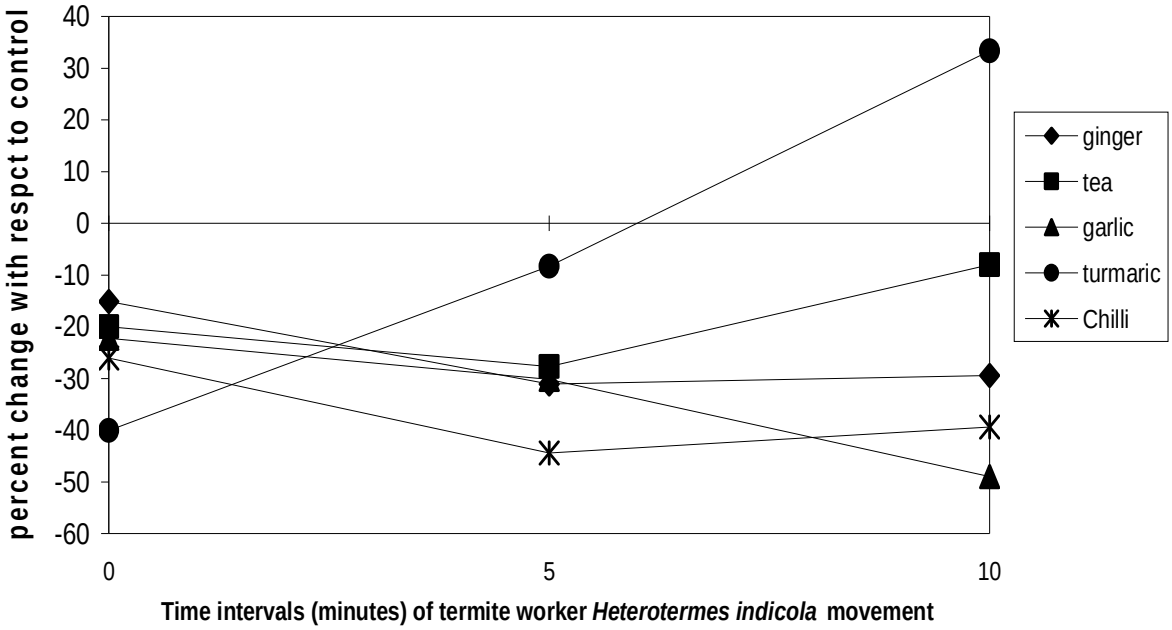


Fig. 3. The effect of different plant extracts on average speed covered by worker of *Heterotermes indicola.*

than control, in black tea 38.22, garlic 29.74 and 44.88% less than control in green chilies. In turmeric extract total distance covered was inflected to 8.55%, which remained almost same up till 10 minutes which is 8.48%. In remaining treatments like in ginger distance covered was increased to 29.90% less than control and in green chilies to 39.06%. In garlic and black tea distance covered

was reduced gradually *i.e.*, 48.14% in case of garlic and 46.32% in black tea.

Figure 3 shows that at 0 min average speed of termite worker in treatment ginger, black tea, garlic, green chilies and turmeric were 15.15, 20, 22.22, 26.08 and 40.10% less than in control, respectively. After 5 min, it reduced to 31, 27.72, 31 and 44.40% in ginger, black tea, garlic and green chilies extract, respectively. In turmeric extract it fluctuated from 8.33% less than control to 33.33% more than control after 10 min. In remaining treatments the average speed also increased. In the case of black tea, it was 8.05% less than control after 10 min while in ginger it was 39.44% and in green chilies 39.41% less than control. In garlic, the average speed reduced to 48.98% less than control. So average speed increased in turmeric, while in garlic it decreased as the time passed. At that time in other treatments like ginger, black tea, green chilies the average speed first decreased and then increased.

Figure 4 shows pause time or time of no movement of termite worker during experiment it was different for all the treatments. The pause of workers in ginger at 0 min was 76.46% longer than control, while in turmeric, green chilies and garlic it was 106.45, 97.44 and 54.93% longer, respectively. In black tea it was 16.93% shorter than control. At 5 min interval significant pause time *i.e.* 223.67% more than control was recorded in ginger extract. In garlic extract pause time increased to 89.68% than control, while in green chilies it was 67.36% higher than control. In turmeric and black tea extract pause time was 6.39% and 38.09% less than control, respectively. At 10 min the pause time was reduced to 81.32% compared with control in ginger extract while in garlic extract, it increased to 155.11% more than the control. In green chilies extract it was 62.03%, while in turmeric extract it reduced to 24.82% and 47.39% less than the control.

So it is concluded that pause time increased in the presence of garlic extract, whereas in ginger extract, the workers first became sluggish but started movement again as the time passed. In turmeric, black tea and green chilies extract movement of termite worker was not effected as time passed.

Figure 5 shows that non-pause time or time of movement almost remains the same during experimental time of black tea extract. No significant change occurred from 0 min to 5 min which was 0.25%, while it increased 1.31% from control at 10 min level. At 0 min in green chilies, ginger, garlic and turmeric extract non pause time was 7.74, 9.04, 10.58 and 13.76% less than control, respectively. As time passed at 5 min level non pause time reduced to 22.35% and 23.25%, respectively in ginger and green chilies extracts. In garlic and turmeric extract it rose to 6.49% and 6.23%, respectively, but it was still less than the control. At 10 min level, movement became fast in ginger and green chilies extract comparing with control that was 12.41% and 8.45%, respectively. In turmeric extract it raised to 14.28% as compared to control. In garlic extract the movement slowed down and at 10 min level it was 16.78% less than control.

## DISCUSSION

Garlic was tested against *H. indicola* in the present study. Only after 24 h 100% mortality occurred in this treatment which is highly significant from other treatments as well as control. In another study of its behavior we noticed that garlic reduced average speed and the total distance covered. Termite worker also became sluggish as the time passed. Park and Shin (2005) reported that garlic oil (3.5 µl/l) caused 100% mortality of Japanese termite, *Reticulitermes spertus* Kolbe after 24 h of treatment. Garlic oil shows the most potent antitermitic activity among the plant essential oils. Iqram *et al*. (2003) carried out experiment on ten different plant extracts and antagonistic fungi for the control of *Xanthomonas compastris*. They found garlic giving the best results against this pest followed by other treatments. Same result was repored by Gareth *et al*. (2006) who used garlic juice for its insecticidal properties against two dipteren pests and compared with insecticide. He concluded that this product is an effective naturally derived insecticide against insect. So garlic should be preferably used in termite control.

Leaf essential oil of turmeric against three species of stored product beetle studied by Tripathi *et al.* (2002) and reported that it reduced the oviposition and egg hatching of all the three pests. This oil totally suppressed progeny production as

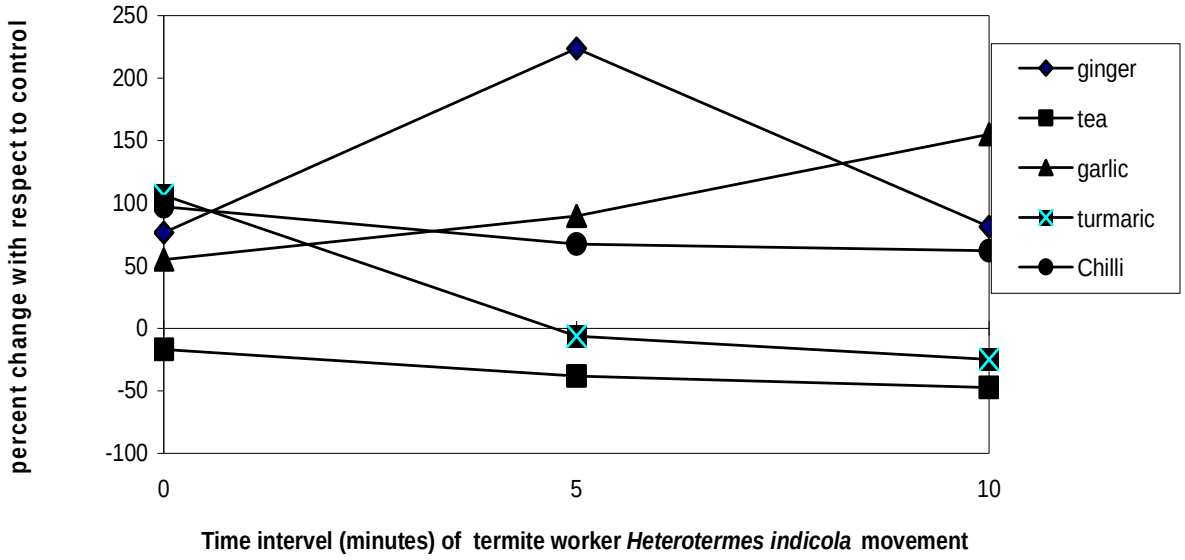


Fig. 4. The effect of different plant extracts on pause time of worker of *Heterotermes indicola.*

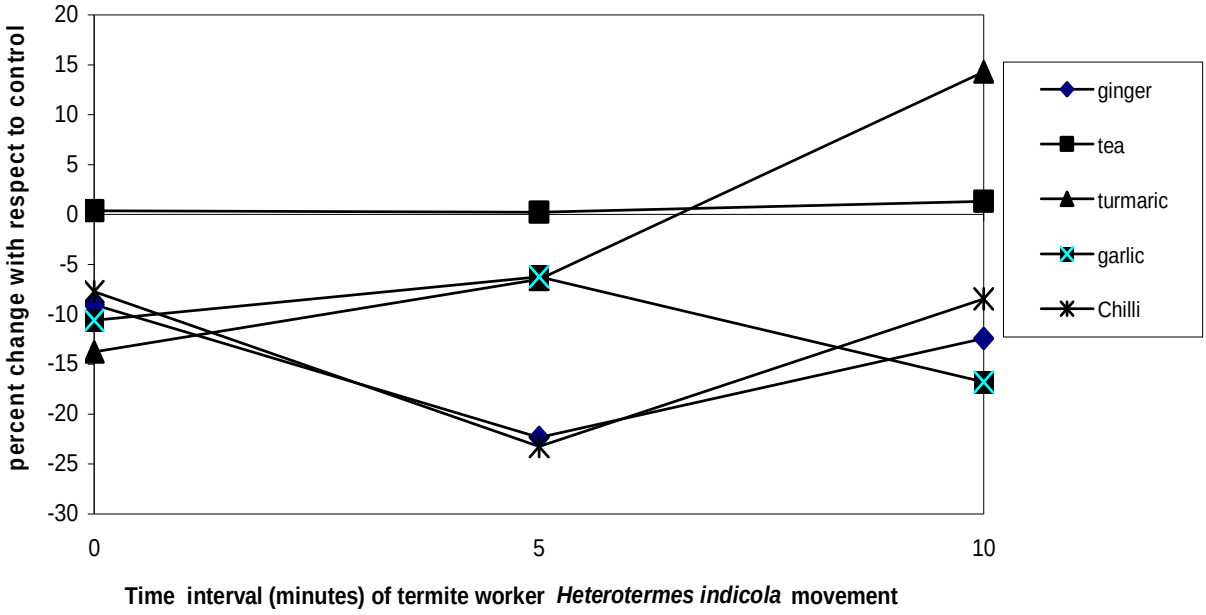


Fig. 5. The effect of different plant extracts on non pause time of termite workers.

concentration increased. In the present study we also found turmeric giving significant result within eleven day of feeding, suggesting the toxicity of this plant against termite. Turmeric contains a mixture of phenolic compounds called curcumin, and a volatile oil with turmerone and zingiberene; cineole and other monoterpenes having insecticidal property. The effect of this plant extract on the behavior of

this specie of termite was also completely different from other plant extract. It became more active or in other words more disturbed and its average speed and total distance covered also increased. So it can be concluded that its behavioral response was completely different from that of other plant extracts. Junwei *et al*. (2008) evaluated turmeric for their larvicidal effect on mosquito which gives safe control.

Ginger extract was also used in this study which gave significant results comparing with control. The repelling property of this product was reported by Wei *et al*. (2004) against white fly (*Bemisia argentifolii*) on tomato. The repellency of this product was attributed with its odor due to which egg laying capacity decreased. The insecticidal property of limonene which is the essential oil of ginger was tested by Ashoka *et al*. (2007) in the form of orange oil against *Coptotermes shiraki*. Significant mortality was found in his experiment only within 5 days. Results of present study suggest that ginger can also be used for its insecticidal activity against termite. Ginger also known for plant diseases control along with insect control. Ayub *et al.* (1997) applied plant extracts for disease (powdery mildew) of mungbean control. According to then the highest dose of ginger gave best control of this disease among all the plant extracts tested.

Carol and Yang (2008) reported tea tree oil with insecticidal property against *Reticulitermes flavipes*. They used 1:50 diluted extract in their experiment and found 95-100% mortality only after one day. In present study only 1:4 diluted extract gave significant results against *H. indicola* as compared to control and other treatments. Mortality can be increased by increasing its concentration and 100% mortality can be achieved as in case of carol and yang experiment. Tea contains theanine and caffeine which have psychoactive properties and effect mental and physical abilities which we also noticed in our behavioral test. Musa (2008) used methanolic extract of African bush tea seeds against cowpea beetle (*Callosobruchus maculatus F*). He found this extract highly effective as compared to control, and persistent in nature for stored grain pests. So it is considered best product not only for control purposes but also for safe storage. Similar types of results were obtained in the present findings.

Vasakorn *et al.* (2004) used ethanol extract of chilies for the control of corn weevil (*Sitophilus zeamais* Motschulsky). Ethanolic chilies seed extract used as solvent was used at different concentration and it gave good results for controlling this pest. Capsaicin is a consituant of green chilies which have burning effect on eater mouth. So it affects behavior of respondent. Present research which was done to find out its insecticidal and behavioral responses coincides with Vasakorn *et al*. (2004) results. Khan (2002) tested different plant extract like garlic and chilies along with other extract for the control of citrus nematode. According to him garlic gives maximum larval mortality. Only after one day of treatment 100% mortality occurred, while chilies also gave satisfactory results after 2 days of application. As time of exposure increased mortality also increased. So findings of Khan (2002) are in accordance with our results. Plant extracts of chilies and garlic along with other plant extracts was also used by Songyot and Ritmontre (2002) for the control of brown plant hopper (*Nilaparvata lugens*). Aslam *et al.* (2004) also applied tea, garlic and red chilies for the control of chickpea beetle (*Callosobruchus chinensis*). They declared garlic as the best control agent. Other plant extracts like tea and red chilies along with other materials also gave good results. Badshah *et al*. (2005) also worked on plant extracts effect on *H. indicola*. Verena and Hertel (2001) also studied some plant extracts for their behavioral impact on termites. He concluded that they reduced the penetrating ability of termite in soil. So these extracts change the behavior of termites as like changing their pause time and non pause time so these findings are in closed conformity with the present study.

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